# Emended description of the genus *Glycomyces* and description of *Glycomyces algeriensis* sp. nov., *Glycomyces arizonensis* sp. nov. and *Glycomyces lechevalierae* sp. nov.

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A polyphasic taxonomic evaluation of presumptive strains representative of the genus *Glycomyces* held within the Agricultural Research Service Culture Collection resulted in the discovery of three novel species. Analysis of the whole cell sugar content of these novel species, as well as of two species presently assigned to the genus, revealed that the whole cell sugar pattern was different from that reported in the formal description of the genus *Glycomyces*. The sugars present in all strains studied included ribose, xylose, mannose and galactose rather than xylose and arabinose as reported in the original description of the genus. Moreover, the menaquinone patterns observed for the novel species also deviated from the original genus description. The formal description of the genus *Glycomyces* is emended to reflect these new data. The novel species proposed and described are *Glycomyces algeriensis* sp. nov. (type strain NRRL B-16327$^T$ = DSM 44727$^T$), *Glycomyces arizonensis* sp. nov. (type strain NRRL B-16153$^T$ = DSM 44726$^T$) and *Glycomyces lechevalierae* sp. nov. (type strain NRRL B-16149$^T$ = DSM 44724$^T$).

The genus *Glycomyces* was described by Labeda *et al.* (1985) and originally comprised two species, *Glycomyces harbinensis*, the type species, and *Glycomyces rutgersensis*. Evtushenko *et al.* (1991) subsequently described *Glycomyces tenuis*, but no additional species have been described, although 16S rRNA gene sequences for putative *Glycomyces* strains are found in the public gene databases. This genus is the sole member of the family *Glycomycetaceae* and it seemed probable that additional species existed in nature. In the course of a phylogenetic evaluation of putative *Glycomyces* strains obtained from the culture collection of Mary Lechevalier and those obtained from attempts at selective isolation of *Glycomyces* from nature, we discovered the existence of three novel *Glycomyces* species.

Two strains, NRRL B-16149$^T$ and NRRL B-16327$^T$, were isolated at the Waksman Institute of Microbiology, Rutgers University, by Mary Lechevalier and were subsequently accessioned into the Agricultural Research Service (ARS) Culture Collection where they have been maintained as lyophilized preparations at 4 °C. A third strain, NRRL B-16153$^T$, was isolated from a soil sample collected from a kangaroo rat (*Dypodymus* sp.) burrow in Arizona. A suspension of 1 g soil in 9 ml sterile tap water was prepared by shaking with two 5 mm glass beads in a 25 × 150 mm test tube at 180 r.p.m. for 30 min. This suspension was subsequently heated at 60 °C for 5 min, and further dilutions were prepared in sterile tap water and spread on the surface of Czapek's sucrose agar (Waksman, 1950) amended with 50 μg each of cycloheximide and nystatin ml$^{-1}$, 10 μg streptomycin ml$^{-1}$ and 25 μg novobiocin ml$^{-1}$. After 3 weeks of incubation at 28 °C, *Glycomyces*-like colonies were observed on several plates. Strains have been maintained as freeze-dried stocks in sterile beef serum at 4 °C since they were added to the ARS Culture Collection.

Chemotaxonomic analysis of the strains for polar lipids, menaquinones and fatty acids was performed using the methods of Grund & Kroppenstedt (1989). Cell wall diamino acid isomer was determined by the method of Staneck & Roberts (1974) and whole cell sugar content was determined by the method of Saddler *et al.* (1991).

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains NRRL B-16149$^T$, NRRL B-16153$^T$ and NRRL B-16327$^T$ are AY462041, AY462042 and AY462044, respectively.

Detailed fatty acid data and menaquinone profiles for the three strains described here are available as supplementary material in IJSEM Online.

Morphological observations were made on the media of Shirling & Gottlieb (1966) and ATCC medium 172 (Cote *et al.*, 1984). Physiological tests, including production of acid from carbohydrates, utilization of organic acids and hydrolysis and decomposition of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, aesculin, urea and hippurate, were evaluated by using the media of Gordon *et al.* (1974). Phosphatase activity was evaluated using the method of Kurup & Schmitt (1973). The temperature range for growth was determined on slants of ATCC medium 172 agar.

Genomic DNA for sequencing was isolated from growth on ATCC medium 172 plates using UltraClean microbial DNA isolation kits (Mo Bio Laboratories, Inc.), amplified and sequenced following previously described procedures (Labeda & Kroppenstedt, 2000). Sequences obtained in this study were manually aligned with actinomycete reference sequences obtained from the public databases using the ARB software environment for sequence data developed by Wolfgang Ludwig and Oliver Strunk (Lehrstuhl für Mikrobiologie, University of Munich, Germany). The program PHYLO_WIN (Galtier *et al.*, 1996) was used to calculate evolutionary distances by the method of Kimura (1980) and to calculate linkages by the neighbour-joining method of Saitou & Nei (1987), using maximum-parsimony and maximum-likelihood analyses. The topographies of the trees resulting from neighbour-joining and maximum-parsimony analyses were evaluated by bootstrap analysis of the data with 500 resamplings. Genomic DNA was isolated and DNA–DNA relatedness between strains was determined spectrophotometrically as described by Labeda (1998) with calculations performed by the method of De Ley *et al.* (1970).

The sequence of the 16S rRNA gene for each of the strains NRRL B-16149$^T$, NRRL B-16153$^T$ and NRRL B-16327$^T$ was determined and deposited in GenBank under accession numbers AY462041, AY462042 and AY462044, respectively. Phylogenetic analysis confirmed that these strains were all members of the genus *Glycomyces*, as can be clearly seen in Fig. 1. 16S rRNA gene sequence similarity between the published sequence for *G. rutgersensis* IFO 14488$^T$ and NRRL B-16149$^T$ and NRRL B-16327$^T$ was 99·93 and 99·60 %, respectively, and similarity between the 16S rRNA gene sequences for NRRL B-16149$^T$ and NRRL B-16327$^T$ was 99·60 %. As a result of these observations, determination of genomic DNA–DNA relatedness between these strains was performed and demonstrated that *G. rutgersensis* NRRL B-16106$^T$ was 54 % related to NRRL B-16149$^T$ and 40 % related to NRRL B-16327$^T$ and that the two latter strains were only 57 % related to each other. These data indicate that strains NRRL B-16149$^T$ and NRRL B-16327$^T$ represent two separate genomic species that are closely related to *G. rutgersensis*. Similarity between the 16S rRNA gene sequences of *G. tenuis* IFO 15904$^T$ and NRRL B-16153$^T$ was observed to be slightly less than 97 %, and therefore genomic DNA–DNA relatedness determinations were not necessary.


**Fig. 1.** Phylogenetic tree of the genus *Glycomyces* calculated from 16S rRNA gene sequence analysis using Kimura's evolutionary distance method (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987). Bar, 0·015 nucleotide substitutions per site.

Chemotaxonomic analytical data for the strains was consistent with membership of the genus *Glycomyces*. The predominant diamino acid present in the cells was *meso*-diaminopimelic acid and the polar lipid pattern for all three strains was identical, comprising phosphatidylinositol mannosides, phosphatidylinositol, phosphatidylglycerol, diphosphatidyl glycerol and similar phosphoglycolipids of unknown composition. Utilization of capillary gas chromatography of alditol acetates of whole cell sugars provided a slightly different sugar profile from that originally reported for this genus based on TLC data. Diagnostic sugars present in all strains were ribose, xylose, mannose and galactose. The fatty acid profiles of these strains consists predominantly of 15-, 16- and 17-carbon iso and anteiso fatty acids. Detailed fatty acid data for the three strains are available in Supplementary Table A in IJSEM Online. Analysis of the menaquinone content of the isolates, as given in the formal species descriptions below, and in Supplementary Table B, proved more interesting; the predominant menaquinone profile was somewhat different from that described in the original genus description, MK-10($H_2$) and MK-10($H_6$), and appeared to be species-specific. It has been reported that *G. harbinensis*, *G. rutgersensis* and *G. tenuis* contain teichoic acids (Potekhina *et al.*, 1993) and that the teichoic acid profiles are diagnostic at the species level (Potekhina *et al.*, 1998), but this analysis was not performed on the proposed novel species. The whole cell sugar and menaquinone profiles observed represent a deviation from the original description of the genus *Glycomyces* and the description must therefore be emended to reflect these revised chemotaxonomic characteristics.

The strains can be differentiated from each other and from recognized species of the genus *Glycomyces* on the basis of a number of physiological characteristics (Table 1). These data, together with both the phylogenetic and the DNA–DNA relatedness determinations, support the designation of these strains as representing novel species of the genus *Glycomyces*, for which the names *Glycomyces algeriensis*

**Table 1.** Differential physiological characteristics of *Glycomyces* species

Strains: 1, *G. harbinensis* NRRL 15337$^T$; 2, *G. rutgersensis* NRRL B-16106$^T$; 3, *G. tenuis* VKM Ac-1250$^T$; 4, *G. algeriensis* sp. nov. NRRL B-16327$^T$; 5, *G. arizonensis* sp. nov. NRRL B-16153$^T$; 6, *G. lechevalierae* sp. nov. NRRL B-16149$^T$. +, Positive; −, negative; W, weak reaction; ND, not determined. Data for *G. tenuis* were taken from Evtushenko *et al.* (1991).

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Hydrolysis of: | | | | | | |
| Adenine | + | + | ND | + | − | + |
| Aesculin | + | + | + | − | + | + |
| Gelatin | − | + | ND | − | − | − |
| Hypoxanthine | + | + | + | − | − | + |
| Starch | + | + | + | − | − | + |
| Tyrosine | − | − | − | − | − | + |
| Utilization of: | | | | | | |
| Acetate | + | + | + | W | − | + |
| Citrate | + | − | − | − | − | + |
| Lactate | − | + | + | − | − | − |
| Malate | + | + | − | W | − | + |
| Mucate | − | − | ND | W | − | + |
| Oxalate | − | − | − | W | − | + |
| Propionate | + | + | ND | W | − | − |
| Succinate | + | − | − | − | − | + |
| Acid from: | | | | | | |
| Adonitol | + | − | − | − | + | + |
| Erythritol | + | − | − | − | + | − |
| Inositol | − | − | − | + | + | + |
| Lactose | + | W | + | + | + | + |
| Mannitol | − | + | − | − | + | − |
| Melibiose | − | − | − | − | − | W |
| Methyl β-xyloside | − | + | ND | − | − | − |
| Raffinose | + | + | + | − | − | − |
| Sorbitol | + | + | − | − | + | W |
| Sucrose | − | − | + | − | + | + |
| Trehalose | + | + | ND | − | + | + |
| Growth at 42 °C | + | − | − | − | − | − |

sp. nov., *Glycomyces arizonensis* sp. nov. and *Glycomyces lechevalierae* sp. nov. are proposed.

## Emended description of the genus *Glycomyces* Labeda *et al.* 1985

*Glycomyces* [Gly′co.my.ces. Gr. adj. *glykus* sweet; Gr. n. *myke* fungus; N.L. n. *Glycomyces* sweet (glycolipid-containing)].

Vegetative mycelia are branching (diameter approximately 0·35–0·40 μm); aerial mycelia may be produced on certain growth media. Oval, spherical or rod-like spores may be formed on the vegetative hyphae in some species; chains of square-ended conidia may be produced on aerial hyphae. Gram-positive. Lysozyme-sensitive. Catalase-positive and aerobic. Type II cell wall composition (*meso*-diaminopimelic acid and glycine) and whole cell sugar pattern consisting of ribose, xylose, mannose and galactose. Type PI phospholipid pattern with significant amounts of phosphatidylinositol mannosides. The menaquinones present predominantly contain 10, 11 and/or 12 isoprene units, but the degree of saturation varies within each species. The type species is *Glycomyces harbinensis*.

## Description of *Glycomyces algeriensis* sp. nov.

*Glycomyces algeriensis* (al.ger.i.en′sis. N.L. masc. adj. *algeriensis* from Algeria, the place of origin of the type strain).

White to yellowish white, waxy, plicate growth on most media. Sparse white aerial hyphae are produced on some media. No soluble pigments are produced. Menaquinones present include MK-10, MK-11 and MK-12. Adenine, allantoin and casein are hydrolysed or decomposed. Aesculin, gelatin, hypoxanthine, starch, tyrosine, urea and xanthine are not hydrolysed or decomposed. Nitrate is weakly reduced. Phosphatase is produced. Acetate, malate, mucate, oxalate and propionate are weakly assimilated. Benzoate, citrate, lactate, succinate and DL-tartrate are not assimilated. Acid is produced from arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, D-mannose, methyl α-D-glucoside, rhamnose, salicin and xylose. Acid is weakly produced from maltose. Acid is not produced from adonitol, dulcitol, *meso*-erythritol, mannitol, melezitose, melibiose, methyl β-xyloside, raffinose, D-sorbitol, sucrose or trehalose. Growth occurs on 5 % NaCl. Temperature range for growth is 15–37 °C.

The type strain, NRRL B-16327$^T$ (=DSM 44727$^T$), was isolated from soil collected in a potato field in Oran, Algeria.

## Description of *Glycomyces arizonensis* sp. nov.

*Glycomyces arizonensis* (a.ri.zon.en′sis. N.L. masc. adj. *arizonensis* from Arizona, the place of origin of the type strain).

White to yellowish white, waxy, plicate growth on most media. No aerial mycelia produced. Faint pinkish–yellowish to yellow soluble pigment produced on some media. Menaquinones present include MK-10($H_2$), MK-10($H_4$), MK-11($H_2$) and MK-11($H_4$). Allantoin, casein and aesculin are hydrolysed or decomposed. Adenine, gelatin, hypoxanthine, starch, tyrosine, urea and xanthine are not hydrolysed or decomposed. Nitrate is weakly reduced. Phosphatase is produced. Acetate, benzoate, citrate, lactate, malate, mucate, propionate, oxalate, succinate and DL-tartrate are not assimilated. Acid is produced from adonitol, arabinose, cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, mannitol, D-mannose, melezitose, rhamnose, salicin, D-sorbitol, sucrose, trehalose and xylose. Acid is not produced from dulcitol, melibiose, methyl β-xyloside or raffinose. Growth occurs on 5 % NaCl. Temperature range for growth is 20–37 °C.

The type strain, NRRL B-16153$^T$ (=DSM 44726$^T$), was isolated from soil collected from a kangaroo rat (*Dipodomys* sp.) burrow in Arizona, USA.

### Description of *Glycomyces lechevalierae* sp. nov.

*Glycomyces lechevalierae* (le.che.val.i.er′ae. N.L. fem. n. *lechevalierae* named for Mary Lechevalier, an American microbiologist who isolated this strain and contributed substantially to the field of actinomycete biology during her career at the Waksman Institute of Microbiology).

White to yellowish white waxy growth on most media. Light grey to yellow plicate growth on ATCC medium 172. Sparse white aerial mycelia produced on several media. Menaquinones present include MK-10, MK-10($H_2$), MK-10($H_4$), MK-11, MK-11($H_2$) and MK-11($H_4$). Adenine, allantoin, casein, aesculin, hypoxanthine, starch and tyrosine are hydrolysed or decomposed. Gelatin, urea and xanthine are not hydrolysed or decomposed. Nitrate is reduced. Phosphatase is produced. Acetate, citrate, malate, mucate, oxalate and succinate are assimilated. Benzoate, lactate, propionate and DL-tartrate are not assimilated. Acid is produced from adonitol, arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, D-mannose, methyl α-D-glucoside, rhamnose, salicin, sucrose, trehalose and xylose. Acid is weakly produced from melibiose and D-sorbitol. Acid is not produced from dulcitol, *meso*-erythritol, mannitol, melezitose, methyl β-xyloside or raffinose. Growth occurs on 5 % NaCl. Temperature range for growth is 15–37 °C.

The type strain, NRRL B-16149$^T$ (=DSM 44724$^T$), was isolated from soil collected from a corn field in Greensburg, PA, USA.

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